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Assessment of Various Gibberellic Acid Concentrations on Seed Germination and Seedling Growth of Chilgoza (*Pinus gerardiana* Wall. ex D. Don)

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Abstract

Chilgoza (*Pinus gerardiana*) forests naturally exist on the highest elevations of the Himalaya Mountains in the provinces of Paktia, Kunar, Nangarhar, Nuristan, and Paktika in the southeast of Afghanistan. These forests provide a sizable source of income for the people living in these areas. The spread and natural regeneration of chilgoza pine forests is declining in the natural environment as a result of overharvesting, erratic annual seed production, and prolonged dormancy; therefore, it is necessary to find an effective method to enhance seed germination and seedling growth. Application of different Gibberellic Acid (GA_3) hormone concentrations (50 ppm, 75 ppm, and 100 ppm), the removal of the seed coat, and control (untreated) treatments were used to conduct an experiment for this study. For each treatment, seedling growth parameters and the percentage of seeds that germinated were examined. The results showed that the T_4 treatment had the highest average length of seedling, shoot, and root, measuring 13.34 cm, 8.1 cm, and 8.4 cm, respectively; whereas the T_1 treatment had the shortest average lengths of seedling, shoot, and root, measuring 11.22 cm, 6.5 cm, and 6.2 cm, respectively. The greatest percentage of germination (78.66%) was observed in T_4 (100 ppm), whereas the lowest percentage (26%) was documented in T_1 (Control). The highest average number of leaves was recorded in T_4 (22.25) and the lowest in T_1 (15.02) treatment. The results indicated that treating chilgoza pine seeds in a 100 ppm solution of GA_3 for 24 hours enhances both the seed germination and sprouting seedling growth.

Keywords

Chilgoza; GA_3; Regeneration; Seed treatment; Seed dormancy

Introduction

The nut tree Chilgoza (*Pinus gerardiana*), commonly referred to as pine nut or edible pine belongs to the *Pinus* genus of the Pinaceae family (Khan *et al*., 2015). According to Luna (2008), Chilgoza pine trees can be found between 1,600 and 3,000 meters above sea level. Compared to coniferous trees, pine trees require less light and water, and they can grow and survive in any environment. It forms a forest community in forests where it grows both alone and in community (Malik, Shamet and Butola, 2012). Chilgoza is an evergreen tree that can grow to a height of 17-27 m, has a trunk thickness of 2-4 m, and lives for 300-400 years on average (Bhattacharyya, LaMarche and Telewski, 1988). The leaves are shaped like needles and have five needles in a cluster, each of which can reach a length of 5 to 15 cm (Kumar *et al*., 2014). The wood of pine trees, which have straight trunks and exfoliated brownish-grey bark, is used for both industrial and ornamental purposes (Richardson, 2000). Chilgoza has a major effect on the socioeconomic growth of the surrounding communities as well as Afghanistan's overall economy. About 120,600 households directly depend on the chilgoza forest for their livelihood; each family earns 444-555 USD annually from this species (Rahman, Salari and Wiar, 2021). The species is degrading due to a lack of natural regeneration, dormancy associated with seeds, and other human causes (Peltier and Dauffy, 2009; Ariez, Salari and Zazai, 2023; Kumar *et al*., 2016). Chilgoza is a significant source of livelihood for people in rural areas where food is scarce and human, wild animal and bird sustainability are threatened. Chilgoza pine production contributed 8% to the global production (Awan and Petennela, 2017). According to Breckle, Hedge and Rafiqpoor (2018), Chilgoza is important for the socioeconomic growth of Afghanistan's rural population. The pine tree is regarded as one of the species that is facing an imminent and serious threat of extinction in Afghanistan as well as some other Asian and European countries. IUCN (2023) has classified the Chilgoza species as near threatened due to grazing, overexploitation, and inherent seed dormancy.

According to Singh *et al*. (2010) and Holl (1999), germination is a complicated process that is influenced by a variety of biological and climatic factors. The extensive collection of pine nuts by locals is the main cause of the very poor natural regeneration of pine (Malik, Shamet and Majid, 2008). Due to pricey edible nuts, pine nut trees have numerous economic advantages, but their widespread use has a detrimental impact on the tree's ability to regenerate because each cone is sought after for maximum profit and no seeds are left over for natural regeneration. Pine nut has issues with dormancy and erratic annual seed production, as a result of which the natural environment experiences a slower germination process (Malik, Shamet and Majid, 2008). Chilgoza is currently propagated only by seed, which shows very low and uneven germination, and slower seedlings growth causing poor natural regeneration. Considering the lack of natural regeneration, it is necessary to protect these forests through artificial regeneration, but to low germination percentage and the high price of seeds are the main barriers to artificial regeneration. Finding a practical way to improve the germination of pine tree seeds is essential because pine trees have difficulty in producing seedlings for reforestation. The ability of various plant hormones to promote seed germination has been investigated by Singh *et al*. (2010). According to Amri (2010) and Ariez, Salari and Zazai (2023), the application of GA_3 can significantly impact seed germination and encourage the vegetative growth of seedlings. Different species of plants responded favorably to growth-promoting hormones, especially GA_3, according to Rawat, Sharma and Ghildiyal (2006) and Bhardwaj (2014), Biochemical changes have a significant effect on seed germination. Studies confirm that increased germination of *Picea smithiana* Boiss., *Abies pindrow* (Royle ex D.Don) Royle, and *Cupressus sempervirens* L. seeds after treatment with gibberellic acid is correlated with either an increase in the production of hydrolytic enzymes (especially amylase) or an increase in embryo development, which is also a result of the stimulation of hydrolytic enzymes (Amen, 1968). The use of GA_3 in seeds increases metabolism, improves germination, and promotes overall plant growth (Rawat, Sharma and Ghildiyal, 2006). In the study by Rawat, Sharma and Ghildiyal (2006), it was found that treating seeds of *Abies spectabilis* (D.Don) Spach, *Picea smithiana* Boiss., and *Cupressus sempervirens* L. for 24 hours with a 100 ppm solution of GA_3 maximized their germination percentage, which was 45%, 57%, and 56% higher than the control, respectively. The type and size of the growth medium are important factors for seed germination and seedling growth. Seed germination and seedling growth are greatly affected by the sowing medium and GA_3 (Ariez, Salari and Zazai, 2023). Based on the study's findings, it can be concluded that gibberellic acid treatment has an impact on seed germination, but the impact varies depending on the plant. The research is designed to determine the best way to address germination issues by examining the effects of various gibberellin concentrations on the germination of pine seeds under local environmental conditions. The prime objective of this research was to ascertain the ideal GA_3 concentration and to examine the impact of various GA_3 concentrations on the germination of pine seeds and on new seedlings growth.

Materials and Method

This research took place at the Research Farm of the Faculty of Agriculture, University of Sayed Jamaluddin Afghani, Afghanistan during 2021-2022. Morphological (qualitative and quantitative) traits were documented at the Research Farm and the research area was marked on map with coordinates, elevations and other details of the chilgoza (*Pinus gerardiana*) prepared neatly**.** The pine seeds originate from the forests that surround the Dewa village of Paron in the Nuristan province, which is located at 35°26′54″ N and 70°55′47″ E. A randomized complete block design (RCBD)[1] was used with five treatments (T_1: control, T_2: 50 ppm, T_3: 75 ppm, T_4: 100ppm, T_5: excision of seed coat) were considered. Three replications of each treatment were conducted, using 50 seeds for each replication and 150 seeds for each treatment. Data were processed and analyzed by using ANOVA and Statistix 8.1 for significance and mean comparison.

Morphological Analysis of Seeds

A digital caliper was used to measure the length, width, and depth of ten randomly selected pine seeds from each treatment before they were planted. The average values were then computed independently. Furthermore, the average weight of fifty seeds sown in one repetition was calculated. Table 1 shows the results of seed dimension.

Table 1: Statistically analysis of seed dimension

Dimensions of seeds	Seed length (mm)	Seed width (mm)	Seed thickness (mm)	Weight of fifty seeds (gram)
Mean size	17.98	6.12	5.18	17.23

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[1] https://online.stat.psu.edu/stat502_fa21/lesson/7/7.3

Pre-Sowing Seed Treatment and Cultivation

Pine seeds were subjected to the five different treatments in an attempt to increase germination and to determine which of these treatments the pine seeds responded to the best. The seeds of each treatment were placed in these concentrations for 24 hours. These seeds were sown in polythene bags over a 10 square meter area after the treatments. Each polythene bag had a 12 cm diameter and a 20 cm length. To avoid seed contamination, the right amount of space between the polythene bags was considered. To prevent the growth of fungi, the seeds were periodically checked and the appropriate measures were taken. The bags were kept wet by routine sprinkler watering. Along with the germination of the seeds, the germination count was periodically carefully documented.

Germination Percentage

The purpose of the seed germination test was to determine the amount of seed germination relative to plant production. Germination percentage in each treatment was calculated from the number of seeds that actually germinated in one growing season and the result was calculated with the help of the following formula.

$$\text{Germination Percentage}=\frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} X\ 100$$

Germination Energy

An indicator of the strength of the seed and seedling, germination energy is a measurement of the rate of germination. The proportion of all seeds in a growing season whose germination was at its highest was used to calculate germination energy and the result was calculated with the help of the following formula.

$$\text{Germination Energy} = \frac{\text{Total number of seeds that germinated up to their maximum number}}{\text{Total number of seeds sown}} X\ 100$$

Germination Mortality

The assessment of germination mortality is a crucial component of seed germination. The reason for the failure of seed germination is the seedling that perishes after germination and fails to develop into a plant. Many times, seeds germinate but fail to develop into strong plants, ultimately dying. The following formula was used to determine the pine seed germination mortality.

$$\text{Germination mortality} = \frac{\text{Number of un−survived seedling}}{\text{Total number of germinated seedling}} X\ 100$$

Characteristics of Seedling Growth

Five seedlings were randomly chosen from each treatment to examine the characteristics of seedling growth. A metal ruler was used to measure the seedling height, stem length, and root length of each seedling and the average number of leaves was calculated.

Vigor Index of Germinated Seeds

Before sowing, the seed vigor index was assessed against various treatments, and the outcome was determined by the following formula.

Seed vigour index = Seedling length x Germination percentage

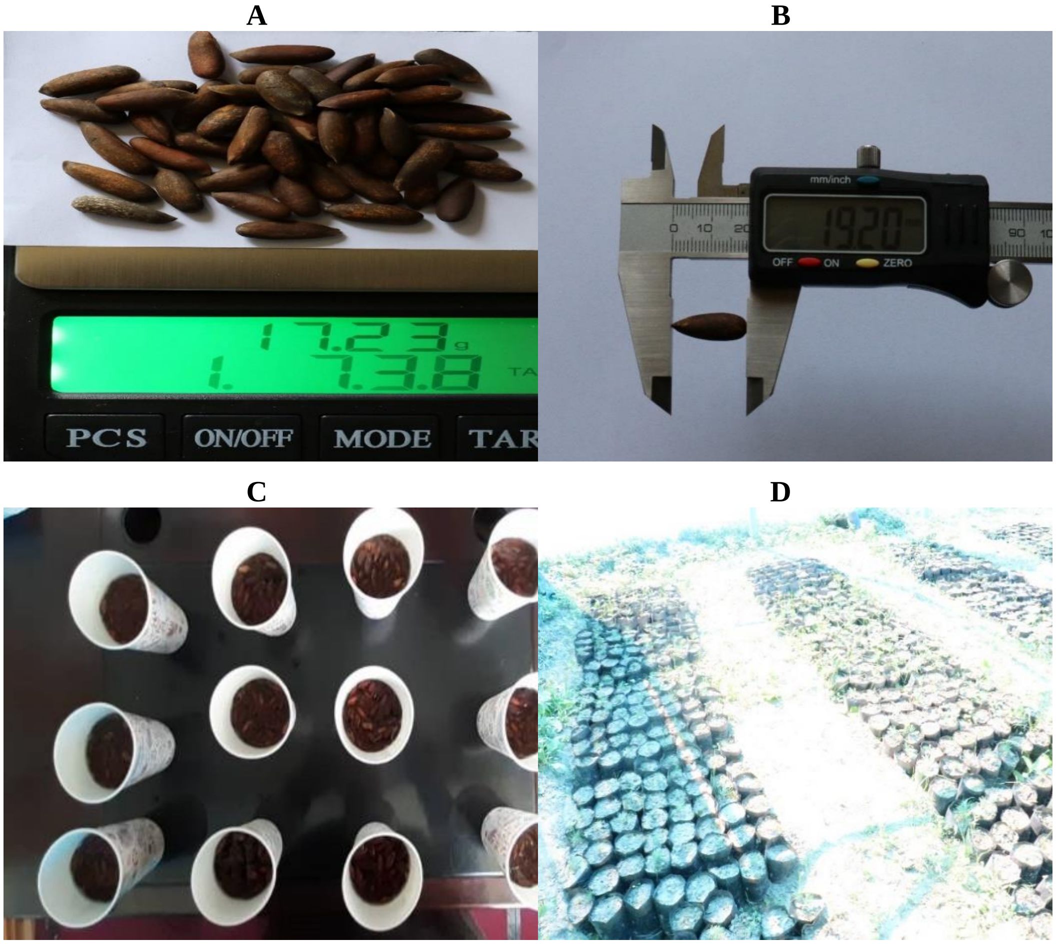


Figure 1: **A:** Measurement of seed weight. **B:** Measurement of seed length with a digital caliper. **C:** Treatment of seedlings with concentrations of GA_3. **D:** Plots of seeds grown in the research farm.

Result and Discussion

Effects of Pre-Sowing Treatment on Seed Germination Percentage

Germination percentage was significantly influence by gibberellic acid concentrations. The germination of the seeds was enhanced using GA_3. The percentage of germination for each treatment and the sum of the three repetitions were calculated. The results obtained are displayed in table 2.

Table 2: Cumulative germination rate and percentage in different treatments and replications

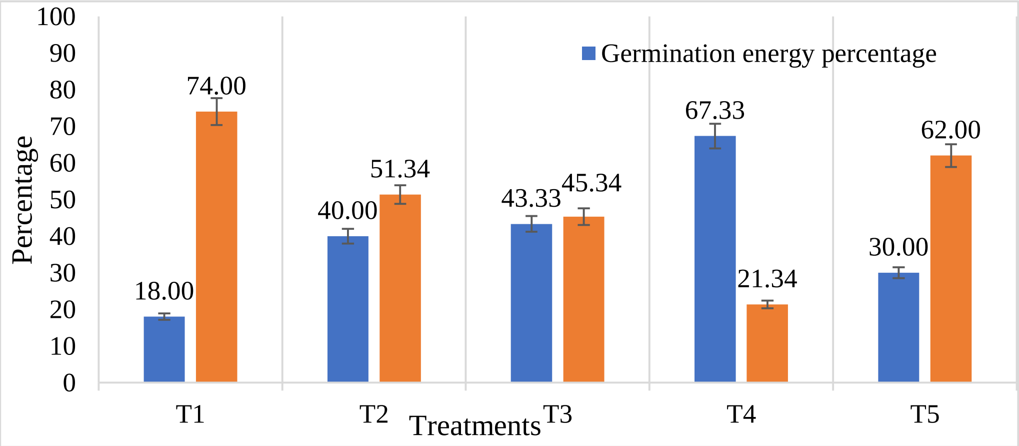
Treatments	*Number of germinated seeds*				*Germination Percentage*
	R_1	R_2	R_3	Mean	
T_1	13	15	11	13.00^{*}	26.00^{*}
T_2	26	22	25	24.33	48.66
T_3	21	32	29	27.33	54.66
T_4	39	41	38	39.33^{**}	78.66^{**}
T_5	22	18	17	19.00	38.00
Mean	24.2	25.6	24	24.60	
CD	6.498				
Sem	1.962				
CV	2.775				

=Highest Germination Percentage, *=** Lowest Germination Percentage

The above table demonstrates that the T_4 treatment was significantly high (39.33) and had the highest positive effect of GA_3 concentrations on the germination of pine seeds (78.66%), with 118 out of 150 seeds germinated followed by T_3 and T_2 as 54.66 per cent and 48.66 per cent, respectively; while the T_1 had the lowest recorded germination rate (26.00 %).

Effects of Pre-Sowing Treatments on Seed Germination Energy and Mortality

The percentage of the total number of seeds at the peak of germination was used to calculate germination energy. After the seeds germinated, the percentage of seedlings that did not survive was also calculated and the detailed amounts are given in graph 1. The analysis of results showed that the germination energy was significantly affected by GA_3 concentrations. The following graph shows that T_4 (67.33%) had the highest calculated germination energy percentage, followed by T_3 (43.33%) and T_2 (40.00%), respectively; while T_1 (18.00%) recorded the lowest germination energy percentage. T_1 calculated the highest percentage (74%) of seed germination failure, followed by T_5 and T_2 (62.00% and 51.34%), respectively; yet T_4 calculated the lowest percentage of germination failure (21.34%).



Graph 1: Effects of pre-sowing treatments on germination energy and mortality percentage of seeds.

Effects of Pre-Sowing Seed Treatments on Seedling Growth Characteristics

To ascertain each seedling's growth characteristics, measurements were taken of the length of each seedling, stem length, root length and the number of leaves. The average results are shown in table 3 below.

Table 3: The average values for various aspects of seedling growth

Treatments	*Average seedlings length (cm)*	*Average stem length (cm)*	*Average roots length (cm)*	*Average number of leaves*
T_1	11.22	6.5	6.2*	15.02*
T_2	11.8 *	7.3	7.1	16.08
T_3	12.12	7.7	7.4	16.02
T_4	13.34**	8.1**	8.4**	22.25**
T_5	12.17	6.4*	7.1	15.05
CD	1.728	0.468	0.980	0.358
Sem	0.522	0.141	0.296	0.108
CV	4.505	3.398	7.081	1.110

**=Highest Average, *= Lowest Average

The collected data demonstrates that the growth parameters of the seedlings are significantly influenced by the various pre-sowing treatments. T_4 had the significantly high and longest average length of seedlings (13.34 cm), followed by T_5 (12.17 cm); while T_1 had the shortest average length of seedlings (11.22 cm). T_4 had the longest average stem length of the seedlings (8.1 cm), followed by T_3 (7.7 cm), and T_5 had the shortest stem length (6.4 cm). Furthermore, T_4 exhibited the largest root length on average (8.4 cm), whereas T_1 had the shortest root length (6.2 cm). The average number of leaves obtained in T_4 (22.25) was the highest, and the average number of leaves obtained in T_1 (15.02) was the lowest across all treatments.

Vigor Index of Germinated Seeds

Table 4: Seed vigor index

Treatments	*Germination Percentage (%)*	*Average Seedling Length (cm)*	*Seed Vigor Index*
T_1	26.00*	11.22*	291.72*
T_2	48.66	11.80	574.18
T_3	54.66	12.12	662.48
T_4	78.66**	13.34**	1049.32**
T_5	38.00	12.17	462.46
CD	0.130	1.728	1.877
Sem	0.039	0.522	0.567
CV	13.815	4.505	16.120

**=Highest, *= Lowest

The percentage of seeds that germinate and the height of the seedling are both factors responsible for the seedling vigor index. This index positively affects the stability of plants and the formation of robust seedlings. Before sowing, the strength index of the seeds was evaluated in comparison to different treatments; more details are given in table 4.

The seed vigor index was significantly high in T_4 (1049.32), followed by T_3 and T_2 (662.48 and 574.18, respectively), and lowest in T_1 (291.72).

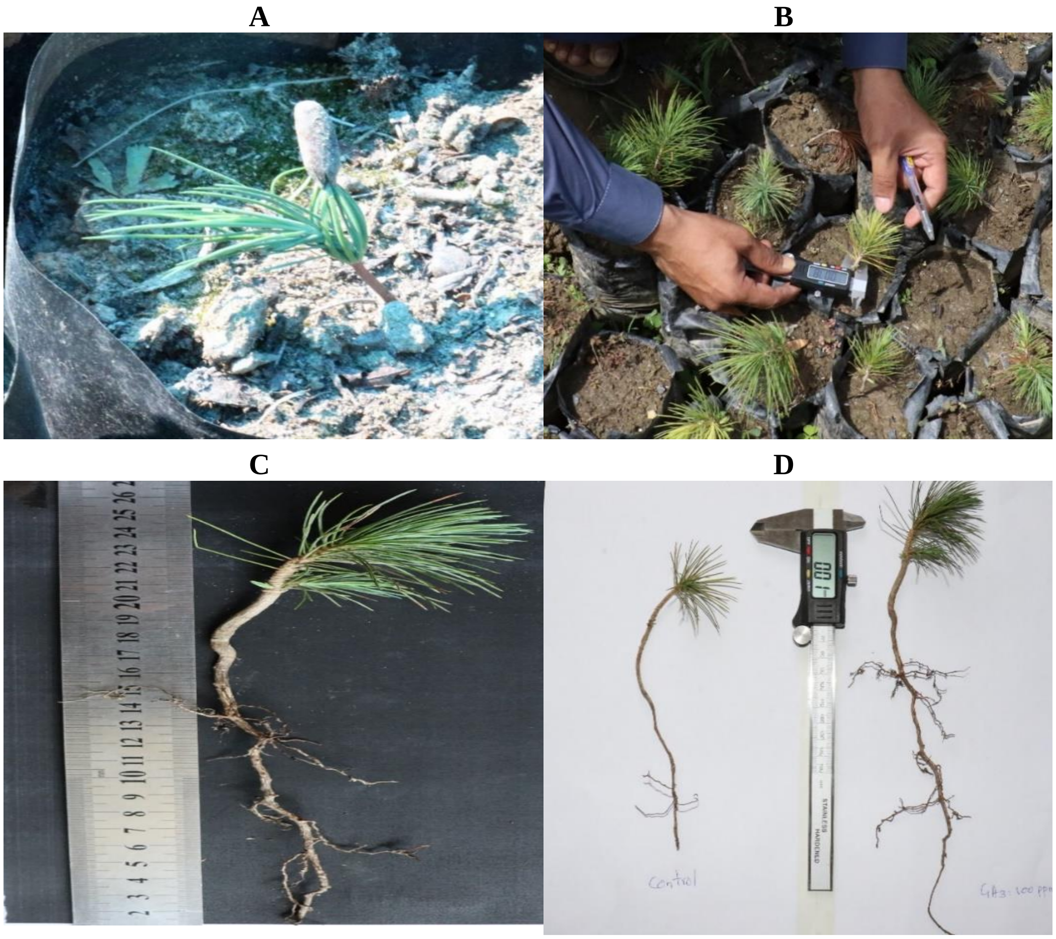


Figure 2: A: Germination of planted pine seeds. B: Measurement of seedling diameter and stem. C: Measuring the seedling length. D: Comparison of control seedling and seedling treated with GA_3.

Discussion

Results of this study showed that various gibberellic acid treatments significantly influenced the germination and seedling growth of the chilgoza pine seeds. The germination percentage and seedling growth had gradually increased with increasing the GA_3 concentration up to 100 ppm. The significantly high germination (78.66) was reported in treatment with T_4 concentration. Similar results were also achieved in the study conducted by Kumar *et al*. (2014) in which the germination was (73.84 and 62.71) percent when seeds were treated with 75 ppm and 150 ppm concentrations of gibberellic acid, respectively. Additionally, these findings contrast slightly from the study conducted by Sharma *et al*. (2019) in which 67 percent of the seeds treated with 100 ppm gibberellic acid. Similar results were also reported by Sharma *et al.* (2020), Breckle, Hedge and Rafiqpoor (2018), and Ariez, Salari and Zazai (2023).

Among the control and other treatments, the seeds treated with a 100 ppm solution of gibberellic acid for an hour produced significantly high percentage of germination energy (67, 33), as shown in T_4. Gibberellic acid, which is produced in the seeds during germination and aids in seed germination, may be the cause of the increase in germination observed with gibberellic acid treatment (Dhoran and Gudadhe, 2012). Gibberellic acid-treated seeds germinated earlier and finished germination faster, which might have made it easier for cytokinins to penetrate the seed coat and reduce or eliminate growth-inhibitory chemicals found in the embryo. As a result, the embryo can sprout by breaking through the seed coat (Çetinbaş and Koyuncu, 2006).

Seedling quality evaluation is essential to the success of reforestation. Even though plant height and stem length are the most frequently assessed parameters during seedling quality evaluation, above-ground plant morphology is not a reliable indication of seedling stability after planting. Davis and Jacobs (2005) stated that the root system's morphological state may improve the probability of transplant success. The increasing of seedling growth might be due to cell enlargement and turgor pressure events stimulated by gibberellic acid (Kumar *et al*., 2014). In the light of the reference of the mentioned authors, different quality characteristics of the seedlings were measured. Significantly high average length of seedlings was recorded in the T_4 treatment as 13.34 cm, and the highest average number of leaves (22.25) was recorded in T_2; the maximum stem and root lengths (8.1 cm) and (8.4 cm), respectively, were also recorded in T_4. The minimum length of seedling, shoot and root was found in T_1 (11.22 cm), (6.5 cm) and (6.2 cm), respectively. These results are in harmony with those of Sharma *et al.* (2019), Kumar *et al*. (2014), Gharib (1972) and Sharma *et al.* (2020).

Abdul-Baki and Anderson (1973) declared that seed treatment is highly significant since it is a sign of healthy plant vigor, and increased plant vigor contributes to stability, making it simpler to grow a sapling into a strong tree. T_4 (1049.32) yielded significantly high and strongest plant index, followed by T_3 and T_2 (662.48 and 574.18, respectively). T_1 has the lowest measured seedling vigor index (291.72). These findings are parallel with the results of Sharma *et al.* (2019) and Sharma *et al.* (2020).

Conclusion

The result of the study highlights the critical challenges facing pine forests, including a decline in distribution and natural regeneration attributed to factors such as excessive pine nut collection, irregular seed production, and prolonged dormancy. The conducted experiments using various treatments, including different concentrations of gibberellic acid and seed coat removal, revealed significant impacts on seed germination and seedling growth. Gibberellic acid treatment emerged as the most effective way in exhibiting the highest germination percentage, germination energy, and the lowest rate of germination failure. The associated seedling growth characteristics, including length, shoot development, root growth, and leaf count, further supported the positive influence of the 100 ppm GA_3 solution. These findings underscore the potential of gibberellic acid, specifically the 100 ppm concentration, as a practical and effective solution for enhancing pine seed germination and fostering robust seedling growth, offering promise for the restoration and sustainability of pine forests in their natural environment.

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Authors' Declarations and Essential Ethical Compliances

Authors' Contributions (in accordance with ICMJE criteria for authorship)

Contribution	*Author 1*	*Author 2*	*Author 3*
Conceived and designed the research or analysis	Yes	Yes	Yes
Collected the data	Yes	No	Yes
Contributed to data analysis & interpretation	Yes	Yes	Yes
Wrote the article/paper	Yes	Yes	No
Critical revision of the article/paper	No	Yes	No
Editing of the article/paper	Yes	Yes	No
Supervision	Yes	No	Yes
Project Administration	Yes	Yes	Yes
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The author(s) solemnly declare(s) that this research has not involved any human subject (body or organs) for experimentation. It was not a clinical research. The contexts of human population/participation were only indirectly covered through literature review. Therefore, an Ethical Clearance (from a Committee or Authority) or ethical obligation of Helsinki Declaration does not apply in cases of this study or written work.

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The author(s) has/have NOT complied with PRISMA standards. It is not relevant in case of this study or written work.

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